Title of Research Project

Pharmacognostical characterization and validation of *Rhus mysorensis* phytoconstituents based formulation for psoriasis.

Introduction: Psoriasis is part complex biological response of body tissue to harmful stimuli, such as damaged cells, or irritants, and is protective involving immune cells, blood vessels, and molecular mediators. Psoriasis appears as drastic cellular changes occur both in epidermis and dermis which narrates to keratinocyte hyperproliferation, there have no proper medication of psoriasis available. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. We have selected a plant for screening of anti-psoriatic activity and will prepare the possible topical formulation.

Pathophysiology of psoriasis: There are many hypotheses and well-evidenced mechanisms are - The activation of pDC is key in starting the development of the psoriatic plaque, and is characterized by the production of type I IFN (IFN-α and IFN-β) [5-7]. LL37–DNA complexes stimulate pDCs through TLR9, LL37–RNA complexes act on mDCs via TLR8 [8, 9]. Activated mDCs migrate into draining lymph nodes and secrete tumor necrosis factor (TNF)-α, IL-23, and IL-12, with the latter two modulating the differentiation and proliferation of Th17 and Th1 cell subsets, respectively.

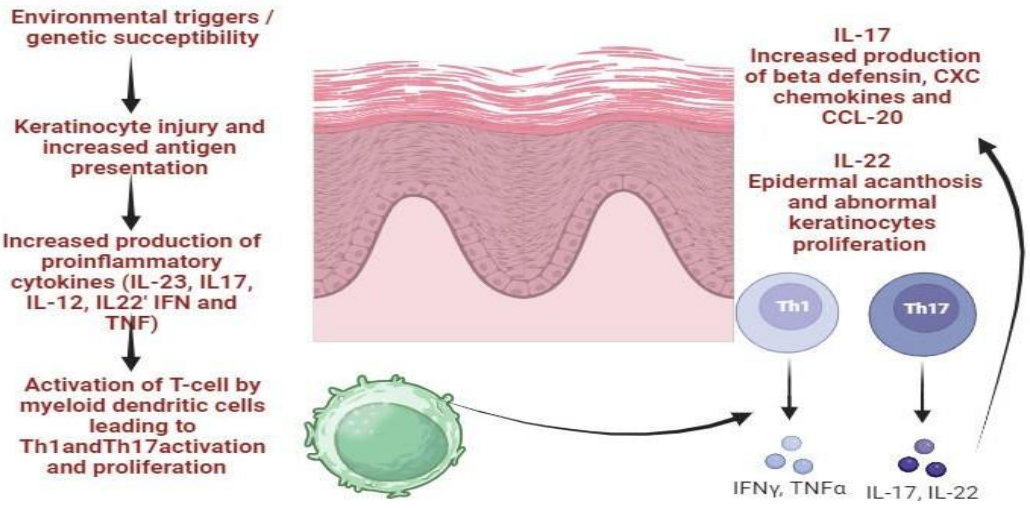

Environmental triggers / genetic succeptibility
Keratinocyte injury and increased antigen presentation
Increased production of proinflammatory cytokines (IL-23, IL17, IL-12, IL22' IFN and TNF)
Activation of T-cell by myeloid dendritic cells leading to Th1andTh17activation and proliferation
IL-17
Increased production of beta defensin, CXC chemokines and CCL-20
IL-22
Epidermal acanthosis and abnormal keratinocytes proliferation
Th1
Th17
IFNγ, TNFα
IL-17, IL-22

Figure 1: Pathophysiology of psoriasis

Tumor necrosis factor-α (TNF-α) is an important mediator in the pathogenesis of psoriasis.Nuclear factor-kappaB (NF-κB) is a transcription factor that regulates the activity of the proinflammatory genes. Psoriasis is an inflammatory disease and the role of TNF-α and NF-κB, should be considerable.

The relationship between NF-κB and TNF-α and their combined role in the development of the characteristic histopathological changes in psoriasis [18].

Plant profile & literature survey

The genus Rhus consists of more than 250 species distributed in the tropics, subtropics andtemperate regions. Synonym: Searsia mysorensis Family: Anacardiaceae Kingdom: Plantae Phylum: Magnoliophyta Class: Magnoliopsida Order: Sapindales Genus: Rhus Species: *Rhus mysorensis*. *Rhus mysorensis* (Dasni) is a medicinal shrubs with thorny branches, family Anacardiaceae. *Rhus mysorensis* comprises a variety of phyto-constituents like glycoside, flavonoids, alkaloids, tannins, saponins, essential oils, markers present are quercetin hydrate, baicalein, fisetin, α-pinene, limonene, sabinene, β-caryophyllene, α and β- eudesmol [1]. *Rhus mysorensis* contain the flavonoids phytoconstituents that possess the individually anti-inflammatory, anti-oxidant and antimicrobial activity [2]. And flavonoids compound i.e baicalein, quercetin, and fisetin possess a good docking score with psoriasis protein [3].

Rationale:

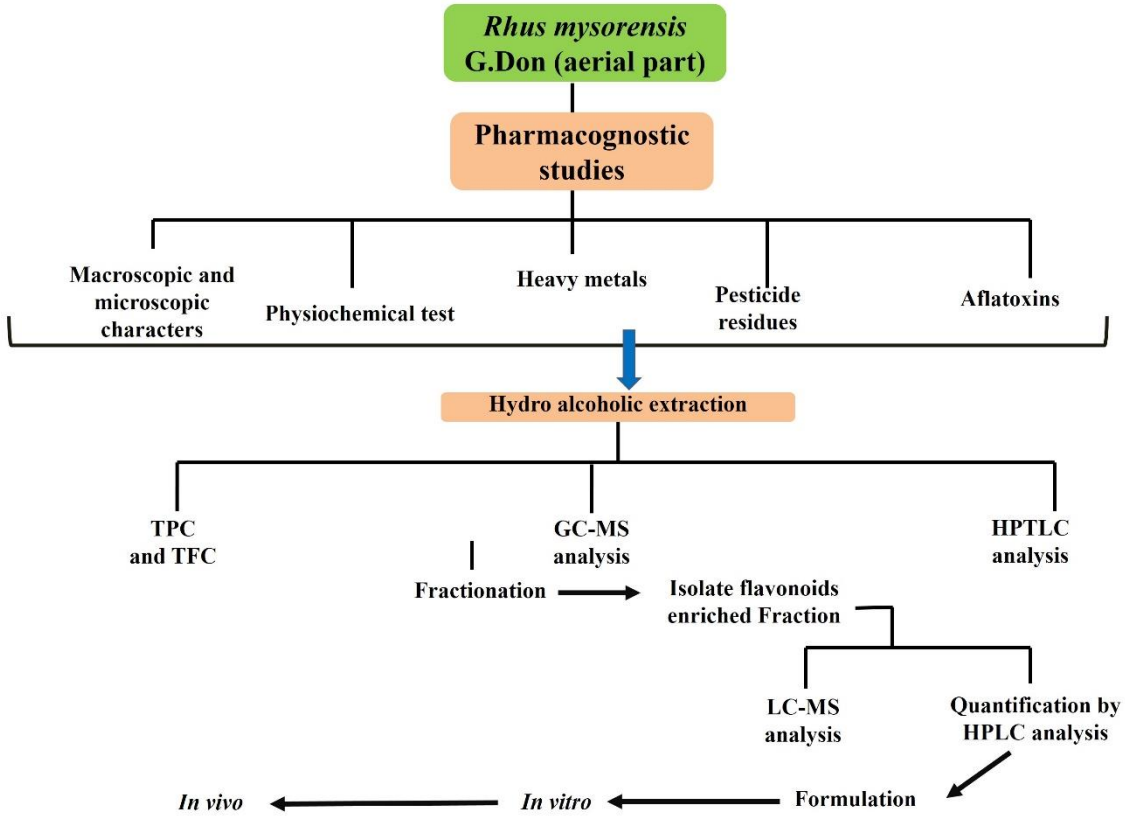
- Psoriasis is a chronic inflammatory, autoimmune, disabling disease for which there is no cure and with a great negative impact on patient quality of life.
- Synthetic compounds have not proper efficacy and they have certain side effects, hence we have select the herbal drug (*Rhus mysorensis*) for antipsoriatic activity according to the mechanism of psoriasis.
- This research initiative aims to explore the potential of natural compounds to provide safer and more effective solutions for managing psoriasis.

OBJECTIVES:

- Prepare the hydroalcoholic extract.
- Perform fingerprinting of the total methanolic extract and essential oils by GC–MS/LC–MS analysis for identification of phytoconstituents.
- Conduct *in-silico* studies of identified compounds for their pharmacological potential.
- Prepare fractions from the total methanolic extract.

- Carry out pharmacological evaluation of the prepared fractions using an *in-vitro* model of psoriasis.
- Develop a formulation using the most bioactive fractions identified from the *in-vitro* studies.
- Perform pharmacological evaluation of the prepared formulation using an *in-vivo* model of psoriasis.

Plan of work:



METHODOLOGY

Plant collection

Fresh young plants of *Rhus mysorensis* will be collected and authenticated using botanical and phytochemical standards. Further these will be deposited in laboratory with your specimen no. for future use.

PHARMACOGNOSTIC STUDIES

Pharmacognostic studies of *Rhus mysorensis* will be described as follows:

Physicochemical Investigation

The physicochemical study of the crude drug will be carried out following ICH guidelines. Evaluating these parameters will be essential for identifying possible adulteration or improper processing of herbal samples. Such assessments will help in understanding the material's characteristics and will support the establishment of suitable quality control standards for herbal drugs. The following tests will be conducted to determine these parameters.

Extractive Value

Extractive value determination will serve as a useful method for evaluating the amount of active constituents that can be extracted from herbal materials, especially when marker compounds are unavailable or when the herb will be used in preparations involving dry residues. Two types of extractive values will be assessed: alcohol-soluble extractive (ASE) and water-soluble extractive (WSE). To perform this analysis, 5 g of powdered sample will be weighed and macerated with ethanol (for ASE) or distilled water (for WSE). The mixture will be shaken continuously for 8 hours and then kept undisturbed for 16 hours at room temperature. The solution will be filtered, the filtrate will be evaporated to dryness, and the extractive value will be calculated using:

$$\text{Extractive Value (\%)} = (\text{Weight of dried extract} / 5\ \text{g of plant material}) \times 100$$

Total Ash Value

The total ash content test will be conducted to detect adulteration and assess purity by quantifying inorganic residues. About 2 g of powdered drug will be weighed into a preheated crucible and incinerated in a muffle furnace at 675 ± 25°C for approximately 3 hours or until a constant weight will be obtained.

After cooling, the percentage of total ash will be calculated as:

$$\text{Total Ash (\%)} = (\text{Weight of ash residue} / 2\ \text{g of sample}) \times 100$$

Acid Insoluble Ash Value

This test will determine the presence of siliceous impurities such as sand and soil. The total ash will be treated with excess 2M HCl, filtered, and the insoluble residue will be incinerated again at 675 ± 25°C until constant weight will be reached.

Acid-Insoluble Ash (%) = (Weight of acid-insoluble residue / 2 g of sample) × 100

Loss on Drying (LOD)

LOD examination will be performed to determine moisture and volatile substances. About 5 g of powdered sample will be placed in pre-dried LOD bottles and heated at 105 ± 5°C for 3 hours. After cooling in a desiccator, samples will be reweighed, and LOD will be calculated:

% LOD = (Weight after drying / Initial weight of sample [5 g]) × 100

All experiments will be performed in triplicate and results will be reported as mean ± SD.

Safety and Contaminant Analysis

Heavy Metal Analysis

Elemental impurity analysis will follow a standard protocol. About 0.25 g of powdered sample will be digested overnight with nitric acid–perchloric acid (3:1). The next day, the mixture will be heated at 180°C until clear. After filtration and dilution with 2% nitric acid, Cd and Pb will be measured by ICP spectrometry, while As and Hg will be detected using a vapor generation unit.

Pesticide Residue Analysis

Pesticide residues will be analyzed using the QuEChERS method (AOAC 2007.01). About 2 g of powdered sample will be mixed with acetonitrile and centrifuged. Cleanup will be performed with $MgSO_4$, PSA, and GCB, followed by GC analysis (GC-7890B, Agilent). Different detectors will be used:

- GC-NPD → organophosphates & carbamates
- GC-μECD → organochlorines & pyrethroids

Temperature programs will follow standard validated methods.

Aflatoxin Analysis

Aflatoxins B1, B2, G1, and G2 will be analyzed using a modified protocol. About 25 g of plant material will be extracted, filtered, cleaned using AflaTest affinity columns, and eluted with methanol. The eluate will be analyzed by UPLC-FLR on a Waters Acquity H-Class Bio System. Mobile phase, filtration, and fluorescence wavelengths will follow validated conditions.

Microbial Count

Microbial quality testing will be conducted according to WHO guidelines. Samples will be diluted, enriched, and plated on appropriate media. Incubation will be carried out at:

- 37°C for bacteria (2–5 days)
- 25°C for fungi (5–7 days)

CFU/g will be calculated, and samples exceeding WHO limits will be considered unacceptable.

Nutritional Value

Nutritional value will be calculated using:

$$\text{Nutritive value (kcal/100 g)} = 4\times\text{protein} + 9\times\text{fat} + 4\times\text{carbohydrate}$$

Carbohydrates will be determined by difference. Protein will be estimated by Micro-Kjeldahl method. Fat will be measured after acid/alkali digestion. Crude fiber will be determined by sequential digestion and incineration.

Preliminary Phytochemical Screening

Preliminary phytochemical screening will be performed to identify major bioactive groups. Standard tests will be carried out for alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, and steroids.

Total Phenolic Content (TPC)

TPC will be determined using the Folin–Ciocalteu method. Gallic acid will serve as standard. Absorbance will be measured at 765 nm, and results will be expressed as mg GAE/g extract.

Total Flavonoid Content (TFC)

TFC will be measured using the aluminum chloride method. Quercetin will be used as standard, and absorbance will be recorded at 510 nm. Results will be expressed as mg QE/g extract.

GC–MS Profiling

The methanolic extract of *Rhus mysorensis* will be analyzed using GC–MS (Shimadzu QP2010S) with an Rxi-5Sil MS column. Helium will serve as carrier gas at 1 mL/min. The temperature program will begin at 60°C and will increase at 5°C/min up to 260°C. Samples will be injected in split mode, and interface/ion-source temperatures will be set at 200°C and 280°C, respectively. This setup will allow precise identification of phytochemicals present.

HPTLC technique:

For the simultaneous estimation of quercetin, baicalein, and fisetin, the HPTLC technique will be developed as per the methods described by Jibril et al. (2019) and Shivraj & Khobragade (2009). Various volumes, specifically 0.1 µL and 0.2 µL of mg/mL standard solutions, will be applied on a pre-prepared HPTLC 60 F254 silica gel plate (20 × 10 cm) as 8-mm bands using a CAMAG Linomat applicator "Linomat 5270409" S/N 270409 (1.00.13) with a 100 µL Hamilton syringe. The application will be performed with inert gas at a delivery speed of 150 nL/s. All sample and standard tracks will be applied as 8-mm bands under a constant flow of nitrogen gas, with the initial application position set at 20 mm and track spacing at 11.4 mm. The plate development chamber ADC2 (CAMAG, Muttenz, Switzerland) will be saturated with 20 mL of mobile-phase vapors for 20 minutes. A total of 10 mL of mobile phase will be used, and the plates will be allowed to develop up to 70 mm from the application point.

The developed HPTLC plates will be examined using the CAMAG TLC Visualizer under 254 nm and 366 nm fluorescence, and documentation will be performed using

VISIONCATS software version 3.1.21109.3. Using the CAMAG TLC Scanner 4 (S/N 270065), the absorption spectra of standard fisetin, baicalein, and quercetin will be recorded in absorption mode within the UV range of 190–400 nm. To detect any possible analyte-specific peaks, the spectral range will be extended below 200 nm. The slit dimensions will be set at 6 mm × 0.45 mm, and the scanning speed will be maintained at 20 mm/s.

LC–ESI–MS/MS Analysis

The ethyl acetate fraction will be analyzed using a liquid chromatography system coupled with electrospray ionization tandem mass spectrometry (LC–ESI–MS/MS) on an Agilent 1290 Infinity II (Model G7129B, USA). Separation will be achieved using a Zorbax C-18 column (150 × 3 mm, 2.7 µm particle size). The mobile phase will consist of solvent A (5 m M ammonium formate in water with 0.1% formic acid) and solvent B (methanol with 0.1% formic acid). A gradient elution will be applied with a flow rate of 0.25 mL/min and an injection volume of 5 µL. The gradient will begin with 80% solvent A and 20% solvent B for the first two minutes, after which the proportions will be adjusted stepwise throughout the analysis. Detection will be performed using a Mass Hunter spectrometer equipped with an electrospray ionization (ESI) source, operating in both positive and negative ion modes. The instrument settings will include a nebulizer gas pressure of 45 psi, a mass detection range from m/z 50 to 2000, a spray voltage of 4 kV, and a column oven temperature of 30 °C. The autosampler will be maintained at 10 °C. Desolvation will be supported by a gas flow of 600 L/h at 350 °C, while sheath gas will be supplied at 0.5 mL/min.

EXPERIMENTAL APPROACH FOR IMIQUIMOD INDUCED PSORIASIS LIKE INFLAMMATION

Psoriasis-like symptoms were induced in Swiss albino mice by applying imiquimod cream topically. The model was developed with modifications based on previously reported protocols. Repeated topical administration of 5% imiquimod cream resulted in epidermal thickening, keratinocyte hyperproliferation, parakeratosis, and inflammatory cell infiltration hallmark features resembling human psoriasis. Imiquimod interacts with Toll-like receptors 7 and 8 (TLR-7/8), triggering the production of pro-inflammatory cytokines like IL-23 and IL-12. These cytokines activate specific T-helper cell subsets (Th-17, Th-22) and result in the increased release of downstream cytokines, including IL-

1β, IL-6, TNF-α, IL-17A, and IL-22, thereby intensifying the inflammatory response characteristic of psoriasis. Adult Swiss albino mice (25–30 g) were randomly assigned to four groups (n = 5 per group): a normal control (NC) receiving vehicle only, a disease control (DC) given 5% imiquimod cream, a group treated with FEF loaded liposomes, and a standard group (SC) administered 0.1% betamethasone valerate cream. Cyclophosphamide at a dose of 3 mg/kg (via intraperitoneal injection) was given for seven days to all animals prior to treatment. imiquimod cream (62.5 mg/day) was applied daily to the right ear of the mice across five successive days, sparing the NC group. On the ninth day, twenty-four hours following the final application, mice were euthanized, and skin samples were collected for subsequent biochemical and histopathological examinations.

Cyclophosphamide pretreatment served as an immunosuppressive measure to simplify the induction of psoriasis, as demonstrated by temporary weight loss and higher susceptibility to imiquimod-evoked inflammation.

Expected Outcome:

The study is expected to identify key bioactive flavonoids from *Rhus mysorensis* with strong anti-psoriatic potential through LC–ESI–MS/MS and *in-silico* analysis. Fractionation and biological screening are anticipated to reveal the most effective ethyl acetate–derived fraction for modulating inflammatory pathways. Development of a nanoformulated topical system is expected to enhance penetration, stability, and sustained release of these compounds. Overall, the project should yield a scientifically validated, plant-based therapeutic formulation with promising efficacy against psoriasis.

References:

1. Wang PW, et al., Therapeutic efficacy of Scutellaria baicalensis Georgi against psoriasis- like lesions via regulating the responses of keratinocyte and macrophage. Biomed Pharmacother. 2022 Nov;155:113798.
2. Fisetin, a 3,7,30,40-Tetrahydroxyflavone Inhibits the PI3K/Akt/mTOR and MAPK Pathways and Ameliorates Psoriasis Pathology in 2D and 3D Organotypic Human Inflammatory Skin Models.
3. Wang PW, et al., Therapeutic efficacy of Scutellaria baicalensis Georgi against

psoriasis- like lesions via regulating the responses of keratinocyte and macrophage. Biomed Pharmacother. 2022 Nov;155:113798.

4. Nestle, F.O., et al., Plasmacytoid predendritic cells initiate psoriasis through interferon- alpha production. J. Exp. Med. 2005, 202, 135–143.
5. Gregorio, et al., Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons. J. Exp. Med. 2010, 207, 2921–2930.
6. Santini, S.M., et al., Interferon-_-conditioned human monocytes combine a TH1-orienting attitude with the induction of autologous TH17 responses: Role of IL-23 and IL-12. PLoS ONE **2011**, 6, e17364..
7. Morizane, S., et al., Antimicrobial peptides in the pathogenesis of psoriasis. J. Dermatol.
   2012, 39, 225–230.
8. Morizane., et al., Cathelicidin antimicrobial peptide ll-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. J. Investig. Dermatol. **2012**, 132, 135–143.
9. Hansel, A., et al., Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong TH17/TH1 T-cell responses. J. Allergy Clin. Immunol. **2011**, 127, 787–794.
10. Chen, L., et al., Skin and gut microbiome in psoriasis: gaining insight into the pathophysiology of it and finding novel therapeutic strategies. Frontiers in Microbiology, 2020. 11: p. 589726.
11. Grice, E.A., et al., The skin microbiome. Nature reviews microbiology, 2011. **9**(4): p. 244- 253.
12. Natarajan, V., et al., *Coexistence of onychomycosis in psoriatic nails: a descriptive study*.
    Indian journal of dermatology, venereology and leprology, 2010. **76**: p. 723.
13. Fry, L. and B.S. Baker, Triggering psoriasis: the role of infections and medications. Clinicsin dermatology, 2007. **25**(6): p. 606-615.
14. Penumala, M., et al., Phytochemical profiling and in vitro screening for anticholinesterase, antioxidant, antiglucosidase and neuroprotective effect of three traditional medicinal plantsfor Alzheimer's Disease and Diabetes Mellitus dual therapy. BMC Complement Altern Med, 2018. **18**(1): p. 77.
15. Sanwar M L., et al., Hypolipidemic and Anti-oxidant Activities of Hydroethanolic Root Extract of Rhus Mysorensis Heyne in Streptozotocin Induced Diabetes in

Wistar Male Rats. Phcog J. 2014; 6(3):62-71.

16. Norull KD., et al., Evaluation of Hepatoprotective activity of Rhus Mysorensis in albino rats. Indian Journal of Research in Pharmacy and Biotechnology. 2014; 2(1):1010-1012.
17. Moorchung N., et al., Expression of tumor necrosis factor-α and nuclear factor-kappaB/RelA and the pathogenesis of psoriasis. Indian J Pathol Microbiol. 2014 Apr- Jun;57(2):205-8. doi: 10.4103/0377-4929.134664. PMID: 24943751.